Claims 3, 4, 12-14 and 18 have been canceled. Claims 1-2, 5-7, 9-11 and 15-17 have been amended to more clearly define the invention. Support for the amendment to claims 1 and 6 can be found in the specification on page 2, lines 6-7, while support for the amendment to claim 9 can be found on page 3, line 8. Claims 2, 5, 7, 10-11 and 15-17 have been amended to as to form. Claims 19-38 have been added. Support for claim 19 can be found in original claim 1 and on page 2, lines 6-7, while support for claims 20-21, 24, 29 and 35-36 can be found on page 2, lines 1-3. Support for claims 22, 26 and 32-33 can be found on page 2, lines 18-19, while support for claims 23, 25, 27-28, 30 and 34 can be found in original claim 5, and page 2, lines 1-20. Finally, support for claims 31 and 37-38 can be found in Examples 1-5. As the amendments to the claims are fully supported by the specification and add no new matter, entry is believed to be in order.

The Examiner has alleged that the present application is not entitled to the benefit of an earlier filing date under 35 U.S.C. § 120. The Examiner recognizes that the Application claims benefit as a continuation of International Application No. PCT/SE95/01235 filed October 19, 1995, assigned U.S. Serial No. 08/809,256, but the Examiner alleges Application Serial No. 08/809,256 was abandoned on July 1, 1997, while the instant Application was filed on November 3, 1997, whereby the applications were not co-pending and the instant application is not entitled to the benefit of the earlier filing date.

The Examiner's attention is directed toward the enclosed copy of the Request for an Extension of Time filed in parent application Serial No. 08/809,256, extending the time for response to a Notification of Missing Parts Requirements under 35 U.S.C. § 371. A copy of the receipt card, indicating receipt of the Request for Extension of Time by the U.S. Patent and Trademark Office as of November 3, 1997 is also enclosed. The Notification of Missing Parts Requirements under 35 U.S.C. § 371, dated June 2, 1997, had a July 2, 1997 due date. In the Request for an Extension of Time filed November 3, 1997, Applicants petitioned for a four (4) month extension, from July 2, 1997 to November 2, 1997, for responding to the Notification of Missing Parts Requirements. As November 2, 1997 was a Sunday, a continuation application claiming priority to Application Serial No. 08/809,256 could be and was properly filed on Monday, November 3, 1997. Thus, the present Application Serial No. 08/963,288 is entitled to the priority date of Application Serial No. 08/809,256.

Application Serial No. 08/809,256 claims priority to International Application PCT/SE95/01235 filed October 19, 1995, and the International Application claims priority to the Swedish Application No. 9403613-4 filed October 21, 1994. Thus, the present application is entitled to a priority date of October 21, 1994. Applicants respectfully request that the Examiner acknowledge the correct priority in the next Official Action.

Claims 11, 16 and 17 have been rejected under 35 U.S.C. § 101 as being directed toward non-statutory subject matter. The Examiner alleges the claims are drawn to an eukaryotic host cell which reads on a host cell *in vivo*. In accordance with the Examiner's recommendation, claims 1, 16 and 17 have been amended to recite "an isolated eukaryotic host cell," whereby the rejection has been overcome.

Claims 1, 2, 5-11 and 15-17 have been rejected under 35 U.S.C. § 112, first paragraph, as not being supported by an enabling specification. The Examiner alleges that the specification does not reasonably provide enablement for the claimed enhancer element, and a method of enhancing transcription *in vivo*, or for methods of enhancing transcription *in vitro* or *in vivo* using any enhancer element comprising the nucleotide sequence TTCTGAGAA and exposing the DNA construct to lactogenic stimuli. The Examiner further alleges one cannot predict whether any and all enhancers comprising the nucleotide sequence would be responsive to lactogenic stimuli, and that transvection experiments are poorly predictive of potential efficiency of a vector in transgenic animals.

As will be set forth below, Applicants submit that claims 1, 2, 5-11 and 15-17 are supported by an enabling specification, whereby the rejection is traversed and reconsideration is respectfully requested.

Claim 1 recites a method of enhancing transcription of a gene in a DNA construct comprising a structural gene for a desired protein or polypeptide and a gene promoter upstream of the structural gene. The method comprises providing upstream of the promoter at least one enhancer element comprising the nucleotide sequence TTCTGAGAA and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

The specification teaches, on page 1, line 29 - page 2, line 5, that the enhancer is a sequence of DNA that confers a response to signals evoked by lactogenic hormones and somatogenic hormones. In Example 1, the specification further teaches how nuclear proteins

which are dependent on growth hormones bound to a 50 bp SPI-GHRE sequence, and by competition with shorter oligonucleotides derived from the 50 bp SPI-GHRE, a core sequence was identified having the sequence TTCTGAGAA. Thus, the specification teaches that an enhancer element comprising a nucleotide sequence TTCTGAGAA functions as a DNA element which is responsive to lactogenic and somatogenic hormones.

The Examiner alleges one cannot predict whether results obtained *in vitro* could be obtained *in vivo*. However, the present specification teaches that the SPI-GHRE sequence functions as a growth hormone regulated DNA element. Yoon et al., *(J. Biol. Chem.*, vol. 256, 19947 (1990)) teach that SPI-GHRE is a segment of the cDNA cloned and discussed by Yoon et al. earlier (*J. Biol. Chem.*, vol. 262, 4284 (1987), cited in Applicants' Information Disclosure Statement of May 26, 1998). In the earlier reference, Yoon et al. disclose that the cDNA is complementary to a rat hepatic mRNA species designated spot 20, and that studies of the cDNA clone and of spot 20 indicate an *in vivo* responsiveness to somatogenic hormones. More specifically, on pages 4288 - 4289 of *J. Biol. Chem.*, 262, Yoon et al. teach that growth hormones act directly on hepatocytes to augment the mRNA, and further teach that the cDNA is induced after growth hormone administration to hypophysectomized rats, indicating that the induction represents a primary event. Thus, one of ordinary skill will appreciate that there is a correlation between *in vitro* and *in vivo* responsiveness of SPI-GHRE to growth hormone.

Therefore, for the reasons discussed above, Applicants submit that claims 1, 2, 5-11 and 15-17 are supported by an enabling specification, whereby the rejection should be withdrawn.

Claims 3, 4, 12-14 and 18 have been rejected under 35 U.S.C. § 112, first paragraph. Claims 3, 4, 12-14 and 18 have been canceled, whereby the rejection has been overcome.

Claims 9 and 10 have been rejected under 35 U.S.C. § 112, first paragraph, as not being sufficiently described in the specification. The Examiner alleges claims 9 and 10 are directed toward an expression vector containing a mammary tissue specific promoter, however, the specification does not contain a written description of the claim expression vector. Claim 9, from which claim 10 depends, has been amended to recite a thiamine kinase promoter as described at page 3, line 8 of the specification, whereby the rejection has been overcome.

Claims 1-6, 8, 10, 12-14 and 18 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner alleges claims 1, 2 and 6 are indefinite in their recitation of "lactogenic stimuli." Claim 1, on which claim 2 depends, and claim 6 have been

amended to recite a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones, and mixtures thereof, whereby the rejection of these claims has been overcome.

The Examiner alleges claims 2, 4, 5, 8 and 10 are indefinite in their recitation of "SPI-grow hormone responsive element (SPI-GHRE)" because it is unclear what the actual boundaries of the element are. The Examiner admits that on page 2 of the specification a 50 bp sequence is identified as the SPI-GHRE but the Examiner alleges that it is unclear as to whether the SPI-GHRE constitutes this entire sequence.

The specification on page 2, lines 14-17 and lines 29-30, specifically states that the sequence of SPI-GHRE is the recited 50 bp sequence. Thus the use of the term "SPI-GHRE" refers to this 50 bp sequence. The specification, on page 2, lines 18-20 and page 3, lines 1-2, uses the term "SPI-GAS" to refer to the shorter 9 bp sequence. The Examiner alleges there is no evidence how much of the 50 bp sequence actually constitutes the responsive element. However, Example 1 of the specification teaches that the 9 bp sequence constitutes the core responsive sequence. The SPI-GAS sequence, which is disclosed as TTCTGAGAA, is located within the larger SPI-GHRE sequence. Thus, claims 2, 4, 5, 8 and 10 are definite, whereby the rejection should be reversed.

The Examiner alleges claim 3 is indefinite with respect to how the expression vectors are provided to animals, and that claims 12-14 and 18 are indefinite in their recitation of "a control sequence". Claims 3, 12-14 and 18 have been canceled, whereby this rejection has been overcome.

Claims 1-2, 9-10 and 16-17 have been rejected under 35 U.S.C. § 102 as being anticipated by Yoon et al., 1990. The Examiner alleges Yoon et al. teach transcription of serine protease inhibitor (SPI 2.1) gene is induced by growth hormone, and that Yoon et al. further teach the isolation and characterization of the SPI 2.1 gene from rat genomic library. The Examiner alleges Yoon et al. teach that portions of the 5′-flanking region of the gene were fused to a heterologous promoter and reporter gene and introduced into rat hepatocytes thereby generating expression vectors and eukaryotic host cells. The Examiner further alleges Yoon et al. teach that a -147 to -102 segment of the gene could confirm growth hormone responsiveness when linked in tandem copies in front of a heterologous promoter.

As will be set forth below, Applicants submit that claims 1-2, 9-10 and 16-17 are not anticipated by Yoon et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

According to claim 1 the invention is directed to a method of enhancing the transcription of a gene in a DNA construct incorporated into the genome of a eukaryotic host cell. The DNA construct comprises a structural gene for a desired protein or polypeptide and a gene promoter upstream of the structural gene. The method comprises providing upstream of said promoter at least one enhancer element comprising the nucleotide sequence TTCTGAGAA, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

The invention is further directed to a method of enhancing the transcription of a gene in a DNA construct comprising a structural gene and a promoter upstream of the structural gene, comprising providing upstream of the promoter at least one enhancer element, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof. According to claim 19, the enhancer element consists essentially of the nucleotide sequence TTCTGAGAA, while according to claim 34, the enhancer element comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence:

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG.

Additionally, the invention is directed to enhancer elements. According to claim 5, the enhancer element comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is not the DNA sequence of SPI-GHRE. According to claim 23, the enhancer element is responsive to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence of the SPI-GHRE.

The invention is also directed to expression vectors. According to claim 8, to invention is directed to an expression vector comprising a structural gene, a promoter, and at least one enhancer element including the nucleotide sequence TTCTGAGAA, with the exception of SPI-GHRE. According to claim 27, the invention is directed to an expression vector comprising a structural gene encoding a structural protein, a promoter, and at least one enhancer element

comprising the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence:

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG.

According to claim 30, the invention is additionally directed to a DNA comprising a promoter, a structural gene, and at least one enhancer element comprising the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence:

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG.

Yoon et al. teach that transcription of the SPI 2.1 gene is induced by growth hormone, and that when portions of the 5'-flanking region are fused to heterologous promoter and reporter genes introduced into hepatocytes, there is a 2 to 3 fold induction of reported gene activity in cells grown in the presence of growth hormone. Yoon et al. also teach that further definition of the essential sequences reveal that a segment from -147 to -102 could confer growth hormone responsiveness, and sets forth the nucleotide sequence for this segment.

Applicants find no teaching or suggestion in Yoon et al. of a segment smaller than the 50 bp segment set forth as SPI-GHRE which is responsive to growth hormone. Yoon et al. disclose 50 to 55, 38 and 32 bp products formed using primer extension and RNase protection methods. However, these groups of products which result from primer extension do not suggest any DNA sequence smaller than the SPI-GHRE set forth in Yoon et al. would confer growth hormone responsiveness to a DNA construct. As Yoon et al. do not teach or suggest that any sequence small than the 50 bp sequence referred to as SPI-GHRE can induce growth hormone responsiveness, there is nothing in Yoon et al. to teach or suggest the use of the 9 bp sequence recited in the claims.

Further Yoon et al. disclose that the SPI gene transcription is induced by growth hormone and teach that, in addition to growth hormone, the pituitary gland produces several other important hormones. To test whether growth hormone itself was sufficient to induce transcription of the gene, Yoon et al. injected growth hormones into hypophysectomized rats, and found that growth hormone itself was capable of inducing nucleotide factor binding to the SPI-GHRE. However, Applicant finds to teaching or suggestion in Yoon et al. of inducing transcription using any other hormones, particularly hormones selected from prolactin, placental lactogen and mixtures thereof, as recited in claims 21, 25 and 35.

Therefore, for the reasons set forth above, Applicants submit that claims 1-2, 9-10 and 16-17, and the remaining claims herein, are not anticipated by Yoon et al., whereby the rejection should be withdrawn.

Claims 1, 2, 5-11 and 15-17 have been rejected under 35 U.S.C. § 102 as being anticipated by Sliva et al., *J. Biol. Chem.* 269 (42): 1628-1624 (Oct. 21, 1994), while claims 1-2 have been rejected under 35 U.S.C. § 102 as being anticipated by Le Stunff et al., *Molec. and Cell. Endocrinology*, 121: 109-117 (1996). As discussed above, the instant application is entitled to a priority date of October 21, 1994. Thus, these references are not proper 35 U.S.C. § 102 references, whereby the rejections should be withdrawn.

Therefore, Applicants submit that claims 1, 2, 5-11, and 15-17 are not anticipated by Sliva et al., and that claims 1-2 are not anticipated by Le Stunff et al., whereby the rejections of these claims should be withdrawn.

For the reasons set forth above, Applicants submit that claims 1-2, 5-11, 13-17 and 19-37 are definite, are supported by an enabling disclosure and are not anticipated by Yoon et al. The Examiner is, therefore, requested to withdraw the rejection of these claims and to allow the application to pass to issue.

Respectfully submitted,
GUNNAR NORSTEDT ET AL.

By Jackie Ann Zurcher

Jackie Ann Zurcher
Registration No.42,251
Attorney for Applicants

DINSMORE & SHOHL
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8377

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